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Optimization of culture parameters of selenium-enriched yeast (*Saccharomyces cerevisiae*) by response surface methodology (RSM)

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Effect of culture conditions (temperature, initial pH value and volume) on the bioaccumulation of selenium (Se) in yeast (*Saccharomyces cerevisiae*) were investigated by response surface methodology (RSM) in this paper. The combined effects of culture conditions on Se yield were studied using a three-level three-factor Box–Behnken design. Fermentation was carried out at different temperature (24–32 °C), initial pH value (4–7) and volume (40–100 mL). The results showed that the optimum conditions for Se enrichment of yeast were found at temperature 27.4 °C, initial pH value 5.8 and volume 89.4 mL. Total Se yield was significantly affected by culture temperature ($P < 0.05$), initial pH value ($P < 0.01$) and volume ($P < 0.01$).

Using a culture medium supplemented with 15 μg/mL sodium selenite (Na_2SeO_3) added at 9 h after inoculation which is the logarithmic growth phase, the maximum biomass and total Se yield in yeast could reach 9.23 g/L and 5.90 mg/L, respectively, which were significantly higher ($P < 0.05$) than that of the control (8.82 g/L and 4.31 mg/L)

1. Introduction

The trace element Selenium (Se) is an essential nutrient for human and animals (Zheng & Ouyang, 2001). This has become increasingly obvious and evidence is growing that Se-enriched derivatives, such as malt (Liu, Zhao, Liu, & Wang, 2006), rice (Hu, Xu, & Chen, 2005) and broccoli (Zeng, Davis, & Finley, 2003), can prevent Se deficiencies and also provide protection against various forms of cancers (Comb, Clark, & Turnbull, 1997; Fleet, 1997). The Se supplementation using microorganisms has received much attention in the past decade. It is known that some microorganisms, especial yeast, under appropriate conditions, can utilize soluble sugars and organic acids, producing biomass with high protein content (Chanda & Chakrabarti, 1996; Chasteen & Bentley, 2003) and meanwhile accumulate large amounts of selenium and incorporate them into organic selenium-containing compounds, mainly selenomethionine, which is the best source of selenium for organisms (Demirci, Pometto, & Cox, 1999; Choi et al., 2002).

Hence, one of the most economic sources of organic forms of Se is yeast grown in quantity in selenium-enriched media (Nève, 1998). It has been found that the water soluble selenium salt (Na_2SeO_3) as a component of the culture medium for yeast producing by conventional batch processing resulted in a substantial amount of Se being absorbed by the yeast (Suhajda, Hegóczki, Janzsó, Pais, & Vereczkey, 2000). In the course of this process the inorganic selenite (low bioavailability, potentially toxic) is converted to safer highly bioactive species with improved nutritional properties.

Therefore, on the basis of optimization of natural culture medium, the present author intended to investigate these parameters and to find the optimal conditions for the accumulation of Se from yeast. Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for designing experiments, establishing models, and analyzing the effects of several independent factors. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple factors and their interactions. Also, study of the individual and interactive effects of these factors will be helpful in efforts to find the target value. Hence, RSM provides an effective tool for investigating the aspects affecting desired response if there are many factors and interactions in the experiment. To determine a suitable polynomial equation for describing the response surface, RSM can be employed to optimize the process (Garrido-Vidal, Pizarro, & González-Sáiz, 2003; Li & Fu, 2005). Box–Behnken design (BBD) is

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an effective technique of RSM for optimizing complex processes. It is widely used in optimizing the conditions. The basic theoretical and fundamental aspects of BBD have been reviewed (Anjum, Tasadduq, & Al-Sultan, 1997; Liu, Huang, & Liao, 1999).

The main factors (temperature, initial pH value and volume) related with total Se yield of selenium-enriched yeast were investigated in this study. The BBD was applied to optimize the parameters of culture conditions, establish a quadratic polynomial regression model. Also, the addition time of Na_2SeO_3 was optimized by a single-factor experiment, so that to find the most significant factor affecting the accumulation of Se and provide scientific basis for industrialization.

2. Materials and methods

2.1. Materials

Yeast (*Saccharomyces cerevisiae*) was obtained from fermentation with a concentration of 15 μg/mL Na_2SeO_3 by comparing the selenium-enrichment ability of 17 strains, which were chosen by resistance screening with a concentration of 50 μg/mL Na_2SeO_3.

Na_2SeO_3 purchased from Sigma was of analytical grade. Na_2SeO_3 standard solution was purchased from Jiangsu center for disease control and prevention. All other chemicals used in this work were also from commercial sources. Germinated brown rice, malt and soybean sprout were bought from the local market.

2.2. Fermentative medium preparation

Germinated brown rice juice (12 Brix), beerwort (12 Brix) and soybean sprout juice (2 Brix) in Fermentative medium were mixed at a ratio of 4:4:2 (Yin, Chen, Gu, & Han, 2009).

2.3. Fermentation conditions

Yeast was pre-grown aerobically for 24 h in PDB (Potato, dextrose, broth) nutrient medium. Fermentative medium was inoculated with 10% (*v*/v) seed liquid and then cultivated for 48 h on a rotary shaker. The starter fermentative conditions of volume, temperature and shaking speed were fixed at 50 /250 mL, 28 ± 0.5 °C and 160 rpm, respectively.

Inorganic Se was added to the sterile medium before the start of yeast cultivation as a solution of Na_2SeO_3, at a concentration of 10 mg/mL. Initial Na_2SeO_3 concentrations in the medium were 0 μg/mL in control and 15 μg/mL in experimental treatments.

2.4. Determination of Se content in yeast

The yeast cells were obtained by centrifugation (1900 g, 10 min). To remove unbound Se, the cells were washed with deionized water thoroughly and then centrifugated at 1900 g for 10 min. The cells were dried under vacuum to a constant weight.

The Se determination was carried out according to the hydride generation atomic fluorescence spectrometry (HG-AFS) method described by Wu, Jin, Shi, and Bi (2007) with some modifications. The instrumental operating conditions were given in Table 1. About 0.2 g dried samples were digested (170 °C, 0.5 h) with 5 mL of a mixture of concentrated HNO_3, $HClO_4$ and H_2SO_4 (v: v: v = 10:4:5) in a digestive flask. Se (+6) was reduced to Se (+4) by addition of 1 mL concentrated HCl. To avoid the volatilization loss of Se, a reflux equipment was employed during digestion process.

After digesting, the solution was cooled and made a constant volume with ultra–pure water. The blank was digested in the same way. The digested product was used for total Se determination. For inorganic Se determination, the biomass mixed with ultra–pure water was extracted in boiling bath for 1 h and made a constant volume. Then the mixture was centrifugated at 8300 g for 15 min and the supernatant liquor was filtrated and the filtrate could be analyzed directly. Organic Se yield was calculated from the difference between the total Se yield and inorganic Se yield.

Table 1
Instrumental operating conditions for HG-AFS.

Parameters	Value
High voltage of PMT (V)	−300
Lamp current (mA)	80
Atomizer temperature (°C)	200
Atomizer height (mm)	8
Gas type	Argon
Carrier gas flow rate (mL/min)	400
Shield gas flow rate (mL/min)	900
Injection volume (mL)	2
Dwell time (s)	1
Read time (s)	10
Read method	Peak area
Measurement method	Stand curve

2.5. Experimental design

To start with, optimization of parameters (temperature, initial pH value and volume) was performed according to Box–Behnken design (Table 2) and a model was developed. Moreover, a single-factor experiment was carried out and the effect of Na_2SeO_3 addition time on Se accumulation was investigated (Table 4).

2.6. Statistical analysis

All trials were carried out in triplicate and all the data were reported as means ± SD (standard deviation). The statistics significance was evaluated using Student's *t*-test and $P < 0.05$ or 0.01 was taken as significant.

2.7. Optimization of fermentation

2.7.1. Analysis of Box–Behnken experiment

By using the software of Design Expert version 6.0.10 (Stat-Ease, Inc.), the data obtained in the Box–Behnken experiment (Table 2) were converted into a second-order polynomial equation with three independent variables:

Table 2
Box–Behnken design according to actual and predicted values of total Se yield.

Trials	X_1 Temp (°C)	X_2 pH value	X_3 Volume (mL)	Total Se yield (mg/L)	
				Observed value	Predicted value
1	−1 (24)	−1 (4)	0 (70)	2.77	2.75
2	1 (32)	−1	0	3.12	3.16
3	−1	1 (7)	0	3.81	3.77
4	1	1	0	3.17	3.19
5	−1	0 (5.5)	−1 (40)	3.75	3.80
6	1	0	−1	3.85	3.83
7	−1	0	1 (100)	4.08	4.10
8	1	0	1	3.94	3.90
9	0 (28)	−1	−1	3.23	3.21
10	0	1	−1	3.78	3.78
11	0	−1	1	3.44	3.44
12	0	1	1	3.89	3.92
13	0	0	0	4.46	4.42
14	0	0	0	4.38	4.40
15	0	0	0	4.42	4.44
16	0	0	0	4.42	4.42
17	0	0	0	4.42	4.42